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Synthesis and Reactions of a Proposed DDT Metabolite, 2,2-*bis*(*p*-Chlorophenyl)acetaldehyde

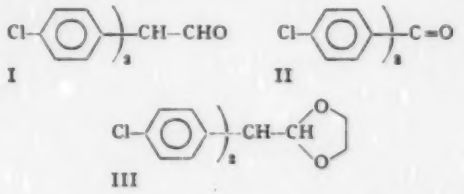
Studies of the metabolism of DDT led to the preparation of 2,2-*bis*(*p*-chlorophenyl)acetaldehyde (I) in order to investigate the possibility of its intermediacy[1] in DDT metabolism. Results of attempts to reproduce a reported synthesis[2,3] of I have shown that both this report and an even earlier report[4] of a different synthetic approach are incorrect. It was observed in this laboratory that I undergoes oxidative deformylation under mild oxidizing conditions to form the benzophenone (II). In comparison, the *o,o'*-isomer was quite stable under the same conditions. These results are of theoretical interest and may have biological significance.

Materials and methods. Melting points were taken on a Fisher-Johns melting point apparatus and are corrected. IR-spectra were obtained with Perkin-Elmer 521 and Beckman IR-12 spectrophotometers. The NMR studies were made with a Varian A-60 spectrometer equipped with a variable temperature probe using TMS or TMS[5] as internal reference. The gas chromatograms (GC) were obtained with a Varian Aerograph 1525 B chromatograph equipped with a tritium detector and employing a polar column of 1% orthophosphoric acid and 3% diethylene glycol succinate as the liquid phase and 30/60 Chromasorb W as the stationary phase. The low resolution mass spectra were obtained with an LKB 9000 gas chromatograph-mass spectrometer (GC-MS). Thin layer chromatograms (TLC) were run on Eastman Chromagrams, Type 6060, with fluorescent indicator and were developed in hexane-chloroform-methanol (3:2:1). The spots were detected with UV-light; Rf (I) 0.59, Rf (II) 0.71. Synthetic methods are given in the following discussion.

Results and discussion. The reported[2] method involved the treatment of 2,2-dichloro-1,1-*bis*(*p*-chlorophenyl)-ethane with sodium hydroxide in refluxing ethylene glycol to form the cyclic acetal, 2-(*p,p'*-dichlorobenzhydryl)-1,3-dioxolane (III). The product obtained by these workers after hydrolysis with refluxing 20% hydrochloric acid and recrystallization melted at 147 °C. Acceptable elemental analyses were given for the product and for its *p*-nitrophenyl-hydrazone (PNPH) derivative, but no spectral data were given.

Our attempts to reproduce the method yielded the crystalline acetal (III) whose structure was confirmed by its IR-, NMR- and mass-spectra. The NMR-spectrum is most diagnostic with a pair of doublets arising from the coupling (J = 4 cps) of the benzhydryl proton (5.82 τ) with the methinyl proton (4.57 τ) of the dioxolane ring.

However, hydrolysis of III gave an oil which could not be crystallized. Thin layer chromatography of the oil indicated that there were 2 major components, neither of which was starting material III. Gas chromatography confirmed that the oil was a mixture of 2 materials which were obtained in variable yields. The low resolution mass-spectrum of each component in the oil was obtained with a GC-MS combination. A study of the spectra indicated that the GC peak with the longest retention time gave a mass-spectrum expected for the desired aldehyde (I).



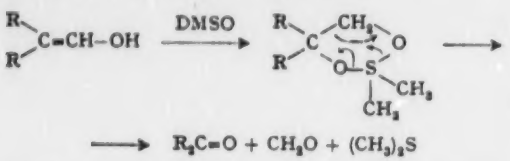
However, the mass-spectrum of the other GC peak had a parent ion which was 14 mass units lower than the parent ion of I and indicated the presence of two chlorine atoms in the molecule. Further analysis of this spectrum suggested that *p,p'*-dichlorobenzophenone (II) was the other major component in the hydrolysis product. An authentic sample of II was obtained and subjected to GC-MS analysis and was found to have an identical GC retention time and superimposable mass-spectrum to that of the unidentified component in the oily hydrolysis product. Crystalline material which had properties identical to those of II was later obtained from the oily hydrolysis product by slow crystallization from methanol. The melting point of authentic II (145 °C) and its PNPH (213 °C) derivative were almost identical to those reported[2] (147 and 211 °C, respectively) for I and its PNPH derivative and indicated that the earlier work was in error.

It was observed that the aldehyde could be separated from II by formation of its bisulfite addition product. Control experiments indicated that the ketone was virtually unreactive toward a saturated sodium bisulfite solution. The aldehyde was obtained as a viscous liquid on decomposition of the addition product with dilute aqueous sodium carbonate solution and was identified by its chromatographic and spectral properties[5]. Vacuum distillation of I is not recommended, since the presence of II was always detectable in the distillate. The thermal

[1] J. E. Peterson and W. H. Robison, Toxicol. appl. Pharmac. *6*, 321 (1964).
[2] R. Riemschneider, I. Arhle, W. Cohnen and E. Heilmann, Chem. Ber. *92*, 900 (1959).
[3] E. F. Riener, U.S. Patent 2,834,708 (1958).
[4] *Beilsteins Handbuch der organischen Chemie*, 4th Ed. *I*, VII, p. 439.
[5] The NMR of I in $CDCl_3$ showed doublets (J = 2.4 cps) at 0.10 τ (CHO) and 5.08 τ (*p*-Cl O_2-CH). The aromatics appeared as a multiplet centered at 3.00 τ.

lability[6] of the aldehyde was further evidenced when repeated injections of I of the same concentration gave varying amounts of II at the expense of I on the acidic polar column used for its GC analysis. The decomposition is probably catalyzed by the orthophosphoric acid additive used to shorten retention times and eliminate tailing.

Attempts to obtain an NMR-spectrum of I in dimethysulfoxide (DMSO) resulted in appreciable decomposition of I into II. This result was confirmed by TLC and GC of the isolated product. However, the electronically analogous 2,2-*bis*(*o*-chlorophenyl)acetaldehyde did not undergo decomposition in DMSO at temperatures ranging from 26–75 °C. Steric hindrance to removal of the benzhydryl proton which is greatest in the *o,o'*-isomer and smallest in the *p,p'*-isomer may explain this result. This steric effect would be manifested if enolization were required to form a reactive species, i.e.



$$\longrightarrow R_2C{=}O + CH_2O + (CH_3)_2S$$

The integrated NMR-spectra of I in d-chloroform and d_6-acetone indicated that I obtained directly from decomposition of the bisulfite addition product has a purity greater than 95%, which was sufficient for subsequent reactions. Aldehyde obtained in this manner was derivatized with *p*-nitrophenylhydrazine according to the method of SHINE[7] and the product was recrystallized from methanol-water as reported earlier[8]. A compound containing one molecule of methanol of crystallization melting at 85–88 °C was obtained and when this product was recrystallized from ether-petroleum ether, the methanol was removed to give a crystalline compound melting at 135–138 °C. Both derivatives were chromatographically identical. The IR-, NMR- and mass-spectra of this product were those expected of the desired aldehyde derivative. When an oily product obtained on hydrolysis of III was treated directly with PNPH according to the reported method, the highly insoluble benzophenone derivative crystallized directly from the reaction mixture while the soluble aldehyde derivative was obtained only after laborious preparative TLC of the mother liquor. These results confirm our belief that the previous workers had isolated ketone instead of I.

Based on these findings, it seemed plausible but mechanistically obscure that I might be intermediate in the formation of II during the hydrolysis of the acetal (III) under the heterogeneous conditions. However, significant amounts of aldehyde were recovered when it was subjected to the conditions of hydrolysis. When the hydrolysis was carried out under homogeneous conditions using *p*-dioxane with aqueous HCl, II was formed as the major product in 1 h at reflux temperature with no TLC evidence for the intermediate formation of I. This result was supported by following the hydrolysis of III by NMR under similar conditions[9].

Although a thorough study of the solvent effects on the mechanism of hydrolysis of III was not made, several solvents were tried in an attempt to improve on the yield of aldehyde. None of these variations in the hydrolysis procedure affording ketone gave a significant increase in the yield of I. An alternative approach to the synthesis of I, which gave fair yields but was more reproducible than hydrolysis of III, was a glycidic ester condensation of II and ethyl chloroacetate. Recent workers[9] also used the Darzen's reaction for the successful preparation of I which was patterned after a successful report[10] of its use to prepare 2-(*o*-chlorophenyl)-2-(*p*-chlorophenyl) acetaldehyde. Aldehyde (I) obtained in our laboratory from this reaction sequence was purified via its bisulfite addition product and was identical in every respect to I obtained via hydrolysis of III.

PERRY et al.[11] in 1965 have found II as an isolable metabolite of DDT which was believed to have been derived from 2,2-*bis*(*p*-chlorophenyl)-acetic acid (DDA). It was observed in our laboratory that DDA does not give rise to II in vitro under the same conditions by which I affords II. On the other hand, I is a logical precursor of DDA. Furthermore, previous work[1] has shown that 2,2-*bis*(*p*-chlorophenyl) ethanol (DDOH) can be obtained from in vivo transformations of other known DDT metabolites. Therefore, the occurrence of II, DDA and DDOH in DDT metabolism is strong supporting evidence for the intermediacy of I.

Greater biological significance may possibly be derived from the inherent reactivity of I as an aldehyde. For example, reaction of I with amino acids would form Schiff bases which might be incorporated into proteins thus chemically binding DDT residues and simulating storage. Therefore, the fate of I depends on whether or not it is enzyme bound and its proximity to stabilizing functional groups which may or may not be bound. However, regardless of what its fate may be, decomposition to form II, oxidation to form DDA, or carbonyl derivatizations with various nucleophiles, its detection in vivo will be difficult because of its instability and reactivity[12].

Zusammenfassung. Es wird eine Methode beschrieben, nach der authentischer 2,2-*bis*(*p*-chlorphenyl)acetaldehyd erhalten wird. Die Charakterisierung dieses Aldehyds hat gezeigt, dass die in der Literatur bisher erschienen Angaben unrichtig sind. Der Aldehyd geht unter milden oxydativen Bedingungen unter Deformylierung in 4,4-Dichlorbenzophenon über. Mögliche biologische Bedeutung dieser Reaktionen werden diskutiert.

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Atlanta Toxicology Branch, Bureau of Science, Food and Drug Administration, Consumer Protection and Environmental Health Service, Public Health Service, U.S. Department of Health, Education, and Welfare, Atlanta (Georgia 30333, USA), 2 May 1969.

[6] A vapor phase IR-spectrum of the aldehyde at elevated temperatures indicated that it apparently undergoes decomposition to *bis*(*p*-chlorophenyl)methane.

[7] H. J. SHINE, J. org. Chem. *24*, 252 (1959).

[8] The hydrolysis was carried out at 73 °C in *p*-dioxane-d_8 with 20% DCl. The NMR was scanned every 5 min for 1 h. The acetal apparently underwent immediate hydrolysis; however, there were no resonances indicating the formation of I. A control experiment had indicated that I was stable under these homogeneous conditions.

[9] R. E. COUNSELL, V. V. RANADE, L. H. LOLA and B. H. HONG, J. med. Chem. *11*, 380 (1968).

[10] T. INOI, P. GERICKE and W. J. HORTON, J. org. Chem. *27*, 4597 (1962).

[11] A. S. PERRY, S. MILLER and A. J. BUCKNER, J. agric. Food Chem. *11*, 457 (1963).

[12] We gratefully acknowledge the assistance of Mr. ROBERT HAWK in obtaining the mass-spectra of the compounds.

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